Study Protocol

Project title:

Determination of DNA repair products in urine after UV irradiation of the skin

Sponsor and principal investigator

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1. **Titel**

Determination of DNA repair products in urine after UV irradiation of the skin.

2. **Purpose**

Problem definition

DNA damage is formed in the skin by sun exposure [1]. The most common DNA damage is called thymine dimers (Figure 1). Many of these injuries are repaired by "Nucleotide excision repair" (NER) and excreted through the urine [2]. The importance of this repair mechanism is seen in patients with defects in their NER, who have a very high risk of developing skin cancer. We want to be able to quantify the amount of thymine dimers in the urine after ultraviolet (UV) irradiation of the skin. With the establishment of such a test system, it will eventually be possible to test potential photoprotective substances or potential photocarcinogenic substances based on damage measured in the urine after a known UV dose. We would like to investigate when the highest secretion of this type of DNA damage occurs using 6 different irradiation regimens. It has previously been possible to quantify thymine dimers in the urine using an isotope method, but that method is no longer available and it is desirable to avoid isotopes [3]. We have developed a new method based on mass spectrometry (UHPLC-MS).

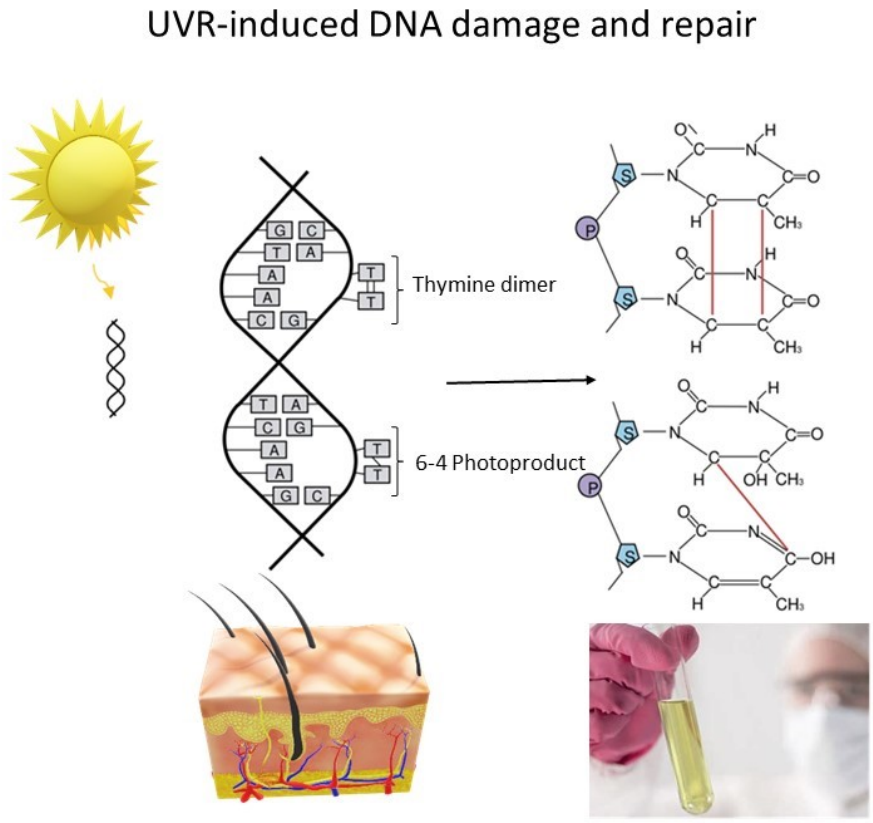

Figure 1. DNA damage of the thymine dimer type occurs by UV irradiation of the skin. The damage will often be repaired and excreted in the urine.

Hypothesis

Our hypothesis is that maximum excretion of thymine dimers in the urine occurs 1-4 days after UV irradiation of the skin.

Endpoints and rationales

The primary endpoint is quantification of thymine dimers in the urine before irradiation as well as every day for up to 7 days after UVR irradiation with six different irradiation regimens in healthy subjects. The 2-3 days where the concentration of excreted thymine dimers is greatest will be the days to collect urine in future studies. One of the six irradiation regimens will consist of healthy volunteers going sun holiday outside of Denmark. The volunteers will receive a UV-dosimeter which measures the UV dose at their location. The urine collection will follow the same procedure as the other groups, upon returning to Denmark.

The secondary endpoints are changes in redness and pigment, measured non-invasively using surface reflectance measurement.

The 6 irradiation regimens will be compared so that the greatest amount of thymine dimers can be determined with the least change in pigment and redness.

Background

Ultraviolet (UV) radiation causes DNA damage in the skin that can eventually lead to skin cancer [1]. Thymin dimer is a DNA damage that is only formed when UV rays hit DNA in the skin cells [1]. Nucleotide excision repair (NER) repairs and removes thymine dimers in the individual cell, which are then excreted in the urine [2]. Thymine dimers in the urine are a biomarker for the total DNA repair[3]. In the past, a method for quantifying thymine dimers in urine using isotope labeling[3] has existed. The method was very sensitive but had a long analysis time and in addition it is an advantage from a work environment to avoid working with radioactive isotopes[3].

We have succeeded in developing a new method of analysis using Ultra High-pressure Liquid Chromatography Mass Spectrometry (UHPLC-MS). This method has high sensitivity and high selectivity while having a fast analysis time. In addition, this equipment is capable of analyzing multiple DNA modifications. Furthermore, there is high sensitivity and high selectivity. This study is a proof-of-concept study in which we would like to investigate when the most optimal time for urine collection is after six different irradiation regimens. The perspective is to be able to use the method to find new potential photo-protective as well as photocarcinogenic substances.

3. **Method**

Study design for healthy subjects

The subjects will be recruited recruited by a notice on Bispebjerg's hospital website and by anthropologist Nynne Johanne Sahl Frederiksen.

For five of the six irradiation regimens the subjects will be divided into 2 groups of 8-10 people (group 1-5). Group 1 is irradiated 3 times with 1 standard erythema dose (SED). 1 SED corresponds to approx. 10 min sun around kl. 13 on a good Danish summer day. Group 2-5 will be irradiated once with up to 3 SED, which corresponds to approx. 30 min around kl. 13 on a good Danish summer day. Group 6 consists of subjects going on sun holiday.

Subjects in group 1-5 are irradiated in a full body UV cabin (Waldmann, Willing-Schwenningen, Germany) with 26 F85 / 100W UV6 tubes (290-350 nm, broad spectrum), TL-01 (290-380 nm, Philips, Eindhoven, The Netherlands) or TL-12 (280-380, Philips, Eindhoven, The Netherlands). Dosages arre

measured out by multiplying the CIE erythema spectrum with the UV-spectra to achieve the same biological effect in the skin. The irradiation time is set on a timer clock, so that the UV cabin switches off after the desired irradiation time. The irradiation time is 13 seconds for 1 SED and 39 seconds for 3 SED. The UV cabin is measured using calibrated equipment, which is also used to measure our UV cabins in the clinic, which are used for patients. The subjects stand up in the cabin and have a screen on so that their eyes and face are not exposed to radiation. When lighting, subjects should only wear underwear, which for men are underpants / boxer shorts, while for women it is bras and panties.

The following groups will be irradiated:

Group 1: 3x1SED in underwear corresponding to approx. 80% irradiated body surface area

Group 2: 1x3SED in underwear corresponding to approx. 80% irradiated body surface area

Group 3: 3x3SED in underwear corresponding to approx. 80% irradiated body surface area

Group 4: 3x3SED wearing shorts and bra/naked torso corresponding to approx. 60% irradiated body surface area

Group 5: 3x3SED wearing shorts and T-shirt corresponding to approx. 40% irradiated body surface area

Group 6: up to 30 subjects who are already planning a sun vacation. The individuals should behave in the sun as they normally do and not do anything differently. The subjects will be provided with a dosimeter, which measures the UV dose at their location. The test subjects will also receive a form where they have to note their clothing/sunscreen consumption. The subjects will be asked 4 questions before they go on vacation (appendix 1) and must undergo a qualitative interview (appendix 2) when they return from vacation and receive their urine test results.

The experiment is performed between October and March in Denmark, to avoid that the subjects do not simultaneously receive UV radiation from the sun and thus can form DNA damage. Subjects must collect morning urine in dispensed containers and must store it in their own freezer until the final visit.

Morning urine samples (2x 50 ml) will be collected before irradiation, labeled as day 1 and up to day 10 for groups 1, 3, 4, and 5, and day 8 for group 2. Group 6 will collect a urine sample in Denmark before the vacation starts and then collect urine as the other 5 groups do when they are back in Denmark. A total of 1000 ml of urine (2 x 50 ml x 10) will be collected for groups 1, 3, 4, 5, and 6, and 800 ml of urine (2 x 50 ml x 8) will be collected for group 2. Before and after each illumination are

measured pigment and redness on the subjects as well as on final visits. Pigment and redness measurement are performed on the back, chest and buttocks.

4. **Statistical considerations**

Previous studies have observed an increase of 1.7 nmol with a standard deviation (SD) of 1.3 nmol [4], with a power of 0.80, as well as significance level of 0.05 in a paired test, then 7 persons must complete the study when we set power to 0.80 and have a significance level of 0.05 and uses the paired test (Power and Sample Size Calculation, PS, Vanderbilt University; version 3.1.2). As the study lasts up to 10 days, dropouts can be expected and therefore 8-10 subjects are included in each of the groups 1-5, as well as up to 30 subjects in group 6. There is an expectation of a larger variability in group 6 due to the varying doses the subjects will receive during their vacation.

5. **Subjects**

A total up to 80 subjects are recruited to participate in the study. Men and women aged 18 or over who meet the criteria for inclusion and exclusion will be included. The subjects are recruited through Bispebjerg Hospital's intranet and by anthropologist Nynne Frederiksen. Subjects will be invited to an initial screening visit where they will receive oral information about the study by trial supervisor Catharina Lerche, Peter Philipsen or Nynne Frederiksen. The meeting takes place in a separate room to ensure a safe and quiet environment. Subjects will be informed that they can bring an assistant if they wish. Written informed consent will be ensured, and takes place at least 24 hours after the screening visit.

Inclusion criteria

Over the age of 18

Written informed consent from the subject.

Exclusion criteria

Immunosuppressed individuals

Sun holiday / ski holiday / solarium 4 weeks before trial start

Skin disease

Medicines that cause photosensitivity or affect DNA repair

Pregnancy

6. Risks, side effects and disadvantages in the short and long term

There is a slight risk of short-term redness after irradiation, as well as after staying in the sun. The UV doses (3 SED) have been chosen so that almost all Danes will avoid redness. The total UV dose corresponds to a ½ hour stay in the sun in the middle of the day during the summer in Denmark. All subjects will be informed both orally and in writing about the risks and possible side effects.

Extraction of new biological material or collection of biological material from already existing biobank

No biobank. The urine samples are analyzed immediately after collection. Excess biological material is destroyed immediately after analysis.

7. **Quality control**

   The Helsinki II Declaration will be respected as well as the standards of good clinical research. Respect for privacy as well as for physical and mental integrity in patients is maintained. The study will be registered at the Knowledge Center for Data Reporting, the Capital Region, in the electronic system Pactius.

8. **Data protection and storage**

   The general data protection regulation will be respected. Research using data from the capital region is considered public research. Use and distribution of data collected in this study will be discussed with patients during the consent process. The project is reported to the Knowledge Center for Data Reporting, the Capital Region of Denmark in the electronic system Pactius. A list of the subjects' names, study ID and date of birth is prepared. All collected data will be anonymised and protected by Danish legislation regarding the handling of personal data and the Health Act. Data are registered and stored for 5 years after completion of the study at the Department of Dermatology, Bispebjerg Hospital.

9. **Economy**

It is Pharmacist Catharina Lerche, Professor Hans Christian Wulf and Engineer Peter Philipsen who have taken the initiative for the experiment and they are all paid by the department of dermatology, Bispebjerg hospital. There is no contribution from companies to this experiment. An external grant of 500,000 DKK has been received from the Danish Research Center For Skin Cancer (https://vfhk.org/en), which is intended to cover the salary for Nynne Frederiksen as well as chemicals for urine analysis. Catharina Lerche has a grant from the Lundbeck Foundation, which covers a taxable compensation of 750-1,500 DKK per participant as inconvenience compensation.

750 DKK will be paid if the exposure is completed and 4 urine samples are provided for groups 1-6. 1,000 DKK will be paid if the exposure is completed and all urine samples are provided for groups 1-5. For group 6, 1,500 DKK will be paid if all urine samples are provided, and both the vacation and interview are completed.

10. **Information for subjects**

    The principle investigator, Catharina M. Lerche, is responsible for providing clear oral and written information about goals, designs and risks of the study, as stated in "Information and consent to participate in health science research projects" by the Ministry of Health and the Elderly. In undisturbed surroundings, the participant will be made aware of their right to have one / or more attendants present, that participation is voluntary and that withdrawal is possible at any time during the study. Subjects are given sufficient reflection time (at least 24 hours). Subjects will be asked to sign a consent form.

11. **Publication of results**

    Positive, negative and conclusive results will be published. The aim is to publish and present the results in a peer-reviewed international dermatology journal and / or at dermatological conferences. The intellectual property rights to the results belong to Bispebjerg Hospital. Publications will comply with the Vancouver Guidelines.

12. **Ethics**

    Personal information and urine samples are processed in accordance with the Personal Data Act and the Health Act. The project is also implemented with minimal health and safety risks for the participants. It is expected that the potential risks of adverse effects in this study are very small. The potential for evidence-based future gains and the future perspectives that this study may provide should be weighed against trial participation. Danish laws on patients' rights and compensation are followed.

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Appendix 1:

Baseline Questions Before Vacation:

1. Have you ever had or do you currently have cancer? 1.1 If yes: Have you ever had or do you currently have skin or melanoma cancer?
2. Do you know people close to you (family, friends, close colleagues, etc.) who have or have had cancer? If yes: 2.1 Who (specify relationship):_____ (e.g., my sister or my friend - Multiple relationships can be mentioned) 2.2 Have any of the above had skin or melanoma cancer?
3. On a scale from 0-10, how significant do you consider your risk of developing skin or melanoma cancer? (0: No risk, 10: High risk)
4. How often do you think about your risk of developing skin or melanoma cancer? (Options: Often, occasionally, rarely, never)

Appendix 2:

Qualitative Interview:

The interviews will revolve around the main questions:

- What happens to the target audience's risk perception when they are informed about UV-induced DNA damage after sun exposure?
- Was it successful in making the abstract risk tangible through this bio-feedback method?

Theme 1: Everyday Life and Relationships: To gain a comprehensive understanding of the informant's world, questions will be asked about their daily life, work, hobbies, family life, etc.

Theme 2: Health History and Habits: To understand what might have influenced the informant's understanding of risk regarding illness, questions will be asked about the informant's health and medical history, current health habits, with a specific focus on sun and tanning bed habits, and behavior during sun vacations. Additionally, questions will be asked about secondary exposure to cancer diseases (especially skin or melanoma cancer) among close relations.

Theme 3: Reactions to Urine Test Results: To investigate reactions to the results of the informant's urine test, questions will be asked about the informant's understanding of the test results, potential intended behavioral changes, and risk perception regarding skin or melanoma cancer.